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Characterization of human peripheral blood innate lymphoid cells

Recently, the family of innate lymphoid cells (ILCs) has come into focus, and are now being recognized as important mediators in establishing tissue homeostasis and as major participants in the initiation and shaping of early immune responses, both in mice and in man. ILCs are cells of lymphoid origin, however they lack any rearranged antigen receptors and are dependent for their development on the transcriptional regulator Id2 and cytokines that signal through the common γ–chain of the IL-2 receptor. They are further described to be negative for lineage markers (CD3, CD19, CD14, CD11c and CD94), while they express the alpha chain of the interleukin (IL)-7 receptor (CD127) and variable levels of cKit (CD117).

In parallel to the subdivision of T helper cells, the family of ILCs can also be divided into three main subsets (Type I, II, III) based on expression of transcription factors and secretion of pro-inflammatory cytokines. Type I ILCs depend on the transcription factor T-bet and secrete mainly interferon (IFN)γ. Like Th2 cells, type II ILCs depend for their development on GATA-3 and secrete type II cytokines such as IL-5 and 13. Type III ILCs are dependent on the transcription factor RORC, express high levels of cKit and secrete IL-22, IL-17 and granulocyte/monocyte colony stimulating factor (GM-CSF). Lymphoid tissue inducer (LTi) cells, which are necessary for organogenesis of secondary lymphoid organs, also belong to this subset of ILCs.

Although a large body of literature has been published on ILCs in recent years, a big portion of this work has been done in murine systems and the characterization of ILCs in adult humans during homeostasis is still lacking. Human ILCs derived from aborted fetal tissue and from surgical waste (tonsils, intestinal biopsies, spleen, skin and lymph nodes) have been studied. However, the number of cells available is limited and it is debatable whether the cells found in these often inflamed, otherwise diseased or fetal tissues represent the cells that are present in the tissues during homeostasis in adult individuals. For this reason we focused our research on ILCs present in adult peripheral blood as a source of ILCs that have the ability to migrate to the different tissues, both in health and disease. Much to our surprise, the ILCs present in peripheral blood (peripheral blood ILCs, PB ILCs) did not exert the same characteristics as described in literature for cells present in epithelial tissues. In

contrast to cells isolated from tonsils, no production of IL-22 and/or IL-17A could be detected by intracellular flow cytometry staining upon activation with IL-7, IL-1β and IL-23 (unpublished observations).
To gain further insight into the biology of PB ILCs, we would like to answer the following questions:

1- Are there activating receptors differentialy expressed on PB ILCs in comparison with ILCs from other tissues?
2- Do PB ILCs preferentialy home to specific tissues (skin, gut, sec. lymphoid organs), and if so, can this homing be correlated with other markers?

Answering these questions will allow better understanding of the development and role of ILCs in homeostasis in humans. Furthermore, alterations from this homeostatic profile may indicate disease development, and function in the future as a biomarker of epithelial diseases. ILCs play a great role in shaping immune responses and possibly also during immune pathology. Understanding the mechanisms that influence these cells might offer new possibilities of targeting these cells during immune disorders or even immunodeficiency.

To answer the above stated questions we will use techniques that have already been established in the lab. Analysis of cells directly *ex-vivo* will be done using flow cytometry. For functional analysis, cells will be sorted. Considering ILC numbers are often too low to perform functional experiments beyond direct phenotyping, cells will be expanded *ex-vivo* using cytokines (stem cell factor (SCF) and IL-7 or IL-2, IL-1β and IL-23), as described by others. Additionaly, ILCs will be grown out from human CD34+ hematopoietic stem cells (HSC), using Notch-ligand expressing OP-9 murine stromal cells with the addition of FLT3L, IL-3 and IL-7. use of recombinant cytokines and antibodies for flow cytometry is thus crucial for this project.